Ph.D. SYNPOSIS

DEVELOPMENT OF THERAPEUTIC INTERVENTIONS FOR MANAGEMENT OF CASUALTIES RELATED TO BURNS AND VENOMOUS BITES

DELHI PHARMACEUTICAL SCIENCES AND RESEARCH UNIVERSITY (DPSRU)

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The 1st Pharmacy University of India

Govt. of N.C.T. of Delhi,

Mehrauli-Badarpur Road, PushpVihar, Sector 3

New Delhi 110017, India

Website: www.dpsru.edu.in

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Accreditation by Life Sciences Sector Skill Development Council

Under the supervision of

Prof. (Dr.) Geeta Aggarwal
Department of Pharmaceutics
DPSRU, New Delhi-110017

Dr. Gaurav Mittal
Scientist 'F'
Life Science Research Board (LSRB)
DRDO, New Delhi

Submitted by:

Ms. Nidhi
Ph.D. Scholar
Department of Pharmaceutics, DPSRU
Registration No.13/Ph.D./DPSRU/2021

BACKGROUND

Combat casualty management is one of the major thrust areas on which INMAS, DRDO is presently working and it focuses on providing first aid and life support solutions to casualties on the battlefield so as to minimize combat related deaths during the golden hour period. Major battlefield associated medical emergencies among military and paramilitary personnel include uncontrolled bleeding, burn related wounds, hypothermia, venomous bites, acute pain, shock, hypovolemia and sepsis or wound infections. Developing safe and effective pre-hospital based therapeutic management strategies during such emergencies is generally more challenging than providing such treatments in a proper hospital set-up because of significant challenges associated during the casualty evacuation process. The present research work has been designed to focus on development of therapeutic interventions for pre-hospital management of two major problems encountered by our military personnel during combat operations, namely, sustaining venomous bites and combat related burn wounds. Consequently, the work is divided into two parts:

Part 1: Development of biopolymeric scaffolds for on-site management of combat related burn wounds

Part 2: Pharmaceutical approaches to augment pre-hospital management of venomous bites and their evaluation using lymphoscintigraphy

Part 1: Development of biopolymeric scaffolds for pre-hospital management of combat related burn wounds

1.1. Introduction

Burn wound injuries sustained on a battlefield due to blast injuries are commonly seen in military and paramilitary personnel engaged in combat operations. Such injuries are associated with significant morbidity and mortality [1]. Globally, it was estimated that approximately 180, 000 deaths are caused every year by burn injuries occurring in low and middle-income countries [2]. On-site management of burn injuries, mainly severe burns (second and third degree), can be challenging to manage and may lead to multi-organ dysfunction/failure due to several inflammatory responses, changes in metabolic function and inadequate supply of blood to the body's tissue and organs. Pre-hospital management of burn patients is therefore one of the major challenges for military personnel during battlefield operations.

Though well- established procedures exist for management of burn wounds in a hospital set-up, no suitable indigenously developed formulation/dressing is available for pre-hospital use during the time an injured soldier is being evacuated to a hospital. Some of these

dressings are imported at an exorbitant cost by the Armed Forces and their availability in required numbers is often a concern [3]. Therefore, there is an urgent demand and need to focus for novel therapeutic interventions that can reduce the temporal gap between pre-hospital care to medical centers in management of burn patients during combat operations.

In recent years, research focusing on scaffolds made up of natural, synthetic, and semi-synthetic polymers has led to the development of newer products for tissue engineering and regenerative medicine. Natural biopolymers are preferable over synthetic and semi-synthetic ones due to their several advantages such as biocompatibility, biodegradability, hydrophilic nature, weak immunogenicity, and mimicing properties of extracellular matrix in wound healing function [4,5]. Several natural biomaterials have been used in for fabrication of various biological dressings such as foams, hydrogels, films and hydro-fibers to facilitate optimal mechanical and cellular properties.

In view of all the above-mentioned aspects, our hypothesis is based on blending of natural biomaterials to produce a potentially active microporous, biopolymeric scaffold dressings with tailored properties that would not only serve as a substrate for skin regeneration but also provide a moist environment to the wound bed during the healing process.

1.2. Literature Review

Historically, burns constitute about 5% to 20% of wounds sustained during military operations [6]. Such injuries are associated with significant morbidity and mortality. However, thermal injuries remain a constant source of mortality and morbidity of warfare casualties. According to literature studies, a great majority of combat burns have occurred due to detonation of improvised explosive devices (IEDs). Other causes of burns include conventional ammunition firing and burns due to landmines, mortars, and rocket-propelled grenades **(Figure 1)** [7].

The combat associated injuries are usually deep burn injuries that are associated with other non burn injuries as well as inhalation injuries [8]. The skin functions as a vital barrier to the external environment and damage of this barrier, as in the case of burns, can disrupt the immune system and makes the body susceptible to infections [9]. Thus infections are a major cause of death after a burn injury as they lead to delayed wound healing and higher mortality [10]. Further, infection leads to the development of heightened immune response, which may result in septic shock. A burn shock is a combination of acute systemic responses, which usually occurs if a thermal injury spreads over a large area of skin (20% TBSA or more) [11].

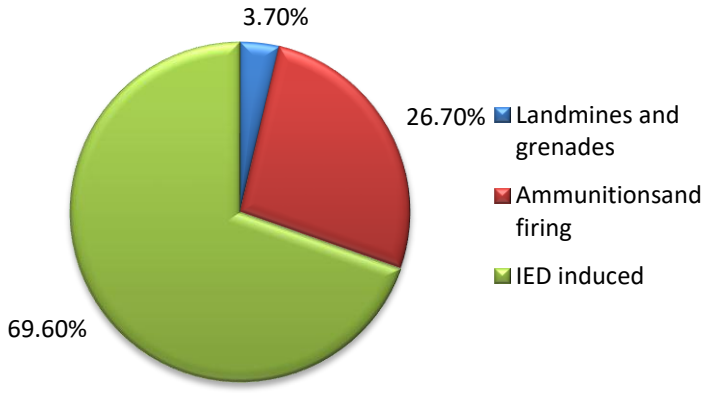

Figure 1: Causes of burns in combat scenarios [81]

A burn shock is characterized by increased capillary permeability, increased hydrostatic pressure across the microvasculature, protein and fluid movement from the intravascular space into the interstitial space, increased systemic vascular resistance, reduced cardiac output, and hypovolemia requiring fluid resuscitation, which can ultimately lead to multi organ failure [12].

1.2.1 Types of burn wounds

Depending on the causative agent, wounds can be classified as below [13]:

a. Physical burns
b. Thermal burns
    - Dry heat burns
    - Wet heat burns
c. Electrical burns
d. Contact burns
    - High voltage
    - Low voltage
e. Flash burns
f. Radiation burns
g. Laser burns
h. 3Chemical burns
    - Acid burns
    - Alkali burns
    - Others

Further depending on the severity and types of tissues affected, thermal burns can be of different degrees **(Figure 2 & Table 1)** [14]:

1. First degree burns or Superficial partial thickness burns
2. Second degree burns or partial degree burns.
3. Third degree or Full thickness burns

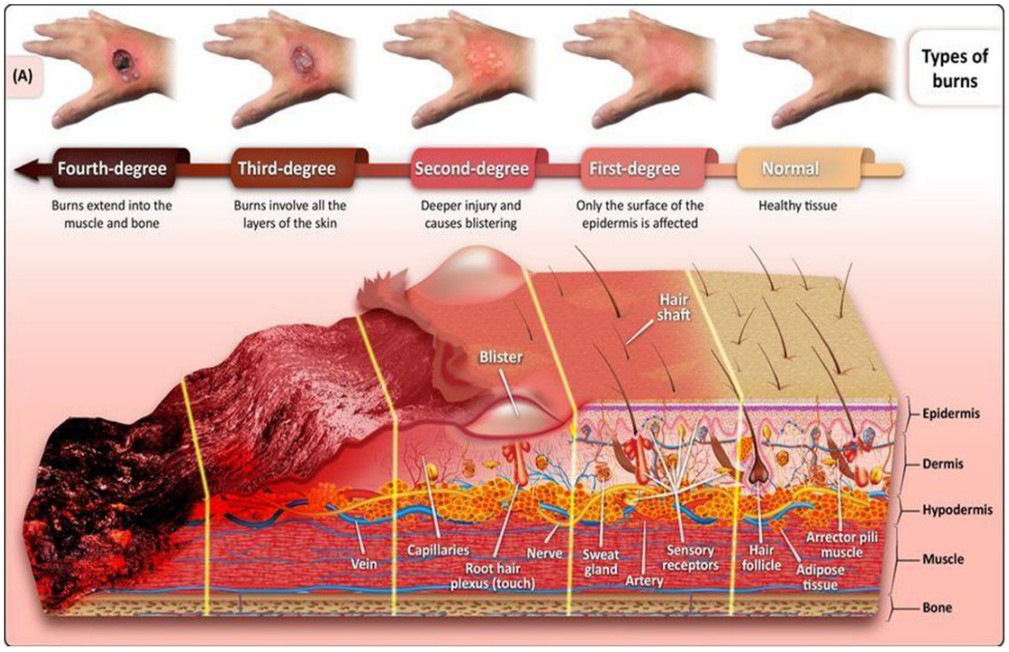

Figure 2: Types of burns in combat operation[14]

Table 1: Classification of burn on the basis of depth**[15]**

S. No.	Type of burn	Tissues affected	Characteristics	Healing time
1	First degree or Superficial burn	Epidermis	Pain, Redness, mild swelling	1 week
2	Second degree or partial (superficial or deep) thickness burn	Epidermis and dermis (Papillary & Reticular)	**Papillary region:** Pain, blisters, splotchy skin, severe swelling **Reticular region:** White, leathery, brelatively painless	3 week 1 month
3	Third degree or Full thickness burn	Deeper tissues (Hypodermi	Numb, white or blackened, charred skin	Does not heal fully without skin grafts (surgical

		s or subcutaneous tissue)		interventions)

1.2.2 Cellular mechanisms and pathological outcomes of healing of burn wounds

Although, burns are different from other wounds in some aspects like degree and depth of inflammation but the cascade of its healing is similar to other wounds [16].The healing process of wounds is subdivided into four major overlapping phases:

A. Hemostasis: When an injury occurs, the initial phase is always an outpouring of lymphatic fluid and blood. In this phase adequate hemostasis is achieved. Both the extrinsic and intrinsic coagulation pathways are activated and play a role in stopping blood loss. Aggregation of platelets follows the arterial vasoconstriction to the damaged endothelial lining. A release of adenosine 5´ diphosphate (ADP) results in the clumping of platelets and initiates the process of thrombosis. This vasoconstriction is a short-lived process that is soon followed by vasodilation, which allows the influx of white cells and more thrombocytes [17].

B. Inflammatory (reactive) phase: Immediately after, the innate immune system of the body initiates the vascular and cellular response to cause inflammation. The neutrophils and monocytes migrate to the site of injury due to the localized vasodilation and extravasation of fluid. In extensive burn injuries, vasodilation increases vascular permeability and leads to massive fluid extravasation, requiring fluid replacement. As the neutrophils start declining, the immune response is sustained by movement of macrophages at these sites via chemokines like kallkireins and fibrin peptides released from coagulation process and substances released from the mast cells like tumour necrosis factor, histamine, proteases, leukotrienes and cytokines. Therefore, the inflammatory phase does does not merely prevent infection during healing, but it also helps in phagocytosis and cleaning of necrotic tissue and toxins released by burned tissue. The duration of the inflammatory stage usually lasts several days [18].

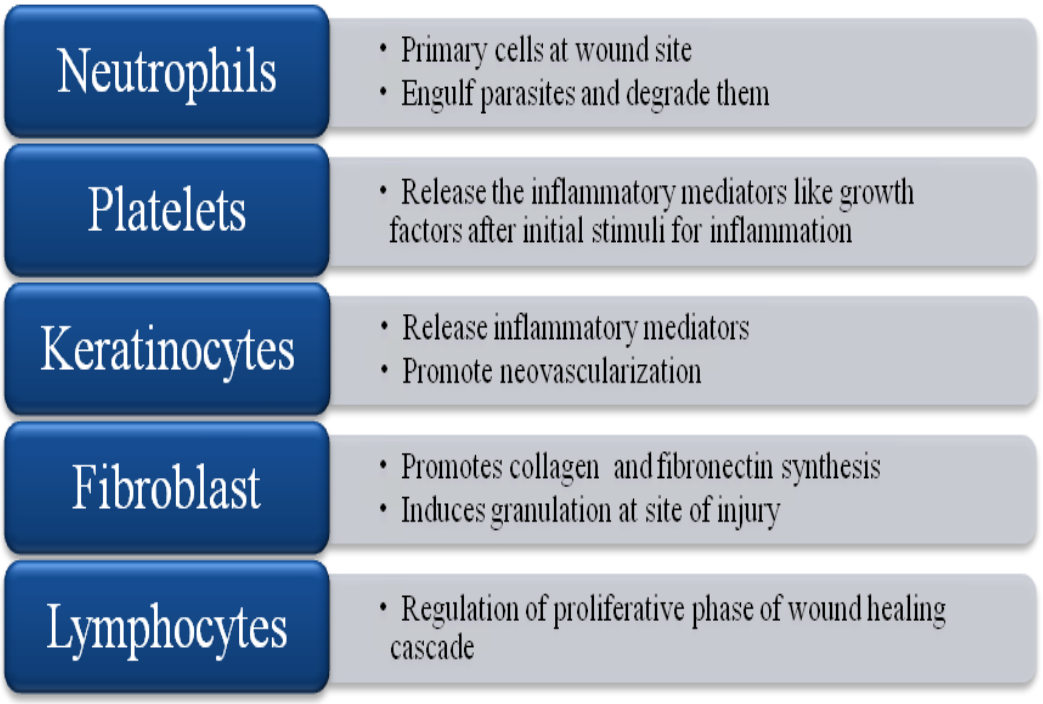
Neutrophils
• Primary cells at wound site
• Engulf parasites and degrade them
Platelets
• Release the inflammatory mediators like growth factors after initial stimuli for inflammation
Keratinocytes
• Release inflammatory mediators
• Promote neovascularization
Fibroblast
• Promotes collagen and fibronectin synthesis
• Induces granulation at site of injury
Lymphocytes
• Regulation of proliferative phase of wound healing cascade

Figure 3: Cells involved in inflammation and their functions[18]

C. Proliferative phase: Following the inflammatory response is the proliferative phase, characterized by formation of granulation tissue, reepithelialization, and neovascularization. Firstly, keratinocytes and fibroblasts are activated by cytokines and growth factors. Further, keratinocytes migrate to the dermis for re-epithelialization of the wound and the vascular network is restored by the fibroblasts. Finally after the re-epithelialization, which takes around 5-7 days, the basement membrane zone is formed between the dermis and epidermis [16,18].

D. Remodelling (maturation) phase: In this phase the wound achieves maximum strength by deposition of fibrous structural proteins i.e., collagen and elastin around epithelial, endothelial and smooth muscle as extracellular matrix [16]. Further in the resolution phase, the ECM remodels into scar tissue and fibroblasts are reformed into myofibroblasts ,that are involved in wound contraction. The balance between the contraction and re-epithelialization regulates the flexibility of the repaired wound [19]. In deep thermal and full thickness burns, the prolonged duration of resolution phase is responsible for hypertrophic scarring and contractures. Finally, the apoptosis of the keratinocytes and inflammatory cells terminate the wound healing process [20].

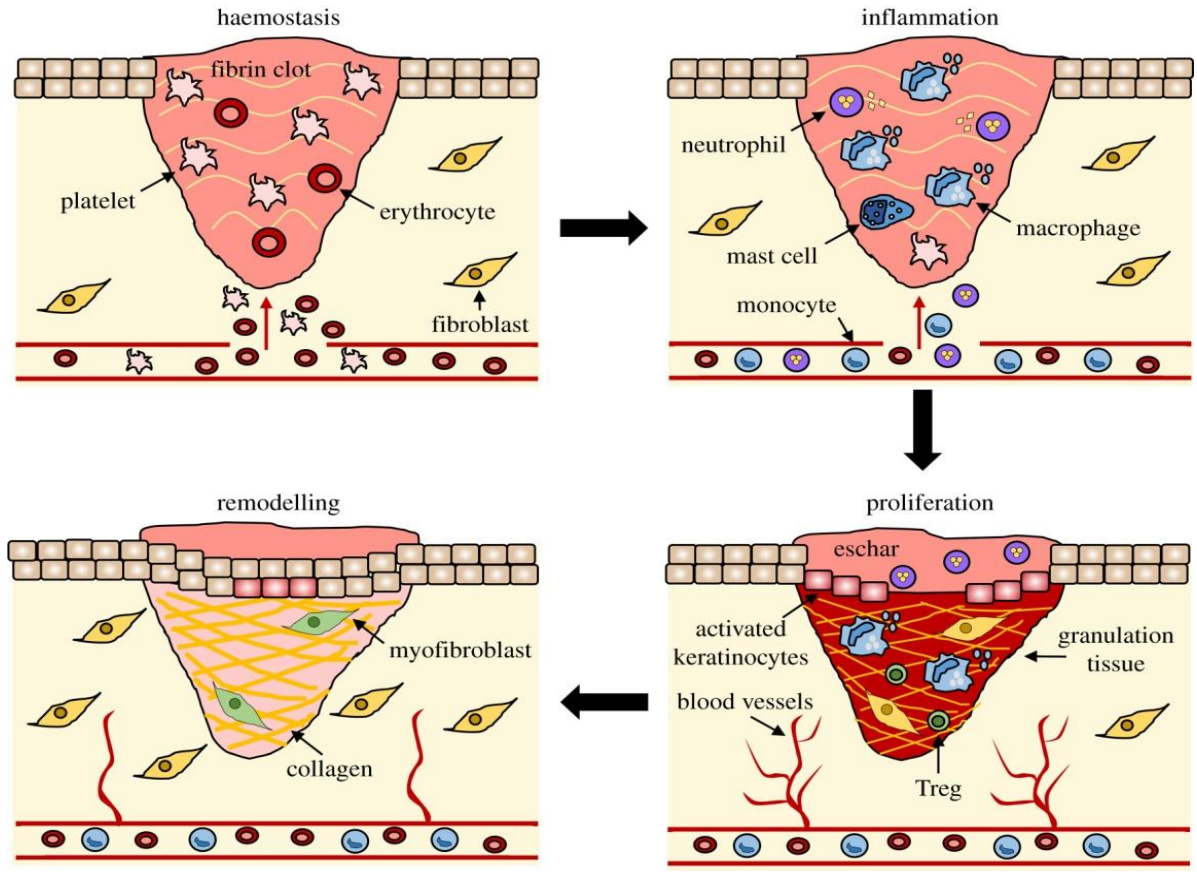

Figure 4: Stages of wound repair and their major cellular components[21]

1.2.3. Natural Biopolymer-Based Scaffolds as wound healing agents

Natural biopolymers have resurged over the past few decades as primary bioactive substances used in the applications of medical materials. Based on their monomeric units and structure, biopolymers are categorized roughly into three classes [22]:

a) Polypeptide- and protein-based: collagen, fibrin, fibrinogen, gelatin, silk, elastin, myosin, keratin, and actin.

b) Polysaccharide-based: chitin, chitosan, alginate, hyaluronic acid, cellulose, agarose, dextran, and glycosaminoglycans.

c) Polynucleotide-based: DNA, linear plasmid DNA, and RNA.

These consist of long chains, including nucleotides, amino acids, or monosaccharides made of repeating covalently bonded groups. Biofunctional molecules which ensure bioactivity, biomimetic nature, and natural restructuring are typically found in such polymers. Bioactivity, biocompatibility, 3D geometry, antigenicity, non-toxic byproducts of biodegradation and intrinsic structural resemblance are the most important properties of natural polymers [23]. Conversely, their key disadvantages, microbial contamination (i.e., endotoxins), decreased tunability, immunogenic reaction, uncontrollable rate of degradation,

and poor mechanical strength restrict their application for hard tissue regeneration. Natural polymers make important contributions to tissue engineering, especially in the manufacture of scaffolds for therapeutic agent delivery. Biopolymeric materials are considered to be a more potent wound healer than synthetic polymers due to their structural arrangement of biopolymers and normal skin physiology are alike and thus they show accelerated wound healing [24,25].

Natural polymers or biopolymers are organic molecules synthesized by the living organisms. Structurally they are a sequence of repeating units/ monomers of amino acids, monosaccharides, nucleotides or esters held by covalent bonds to form peptides, polysaccharides, polyphenols or polyesters, etc. Biopolymers of diverse origin such as plant(starch, cellulose, carboxymethyl cellulose (CMC), and natural rubber), animal (Gelatin, hyaluronic acid, chitosan), fungi (Pullulan), bacteria (bacterial cellulose, exopolysaccharides) and algae (alginate) are used in based on their properties. These polymers offer advantage over the synthetic materials because of their biocompatibility, biodegradability, lower antigenicity and renewability [23].

Biopolymers have material properties that allow them to be easily moulded into hydrogel, scaffold and blend with other polymers which offer multiple benefits with increased mechanical strength, biomimetic properties containing cell-specific domain and several other desired features for the development as skin substituent [23]. They can possess either of antibacterial, anti-inflammatory, proliferative or other targeted actions to specific cells, thus playing a key role in wound healing process. The various types of biopolymers used in skin and tissue engineering are shown in **(Table 2)**.

In addition biopolymeric formulations have become the object of extensive research due to their unique features that make them suitable for a broad range of topical applications. A vital role has been played by scaffolds in the biomedical field, mainly in context of tissue engineering and wound dressings. Scaffolds in general can be defined as highly hydrophilic three-dimensional polymeric networks in lyophilized forms [20]. Due to their specific structural networking, scaffolds have the ability to absorb and swell when immersed in biological fluids like blood, exudates, serum, saline etc. They trap the liquid molecules in their interstitial spaces in quantities up to thousand times of their dry weight.

Table 2: Different types of biopolymers used in skin and tissue engineering

Many biopolymers constituting these scaffolds have the ability to absorb and swell, besides being biodegradable in nature [16,21]. The bonding of biopolymers may rely on physical

Biopolymer	Source (origin)	Monomer units	Linkage	Characteristics	Biological roles	References
1. Hyaluronic acid (or hyaluronan, HA)	Animal origin (fluids in the eyes and joints)	d-glucuronic acid and N-acetyl-d-glucosamine	β (1 → 4) linkage and α (1 → 3) linkage.	Biocompatible, bacteriostatic, non-antigenicity, viscoelstic property, hygroscopic, antioxidant, anti-inflammatory and anti-oedamatous	Stimulates fibroblasts and keratinocytes proliferation and migration, anti-inflammatory, soft tissue growth, prompts your body to make more collagen and elastin, keeps your skin moisturized, prevents tightness, boots elasticity, and reduces scarring.	[26]
3. Alginic acid	Brown algae Laminaria, Ascophyllum and Macrocystis sps. (found in seaweeds as salts of different metals, primarily sodium and calcium)	β-d-Mannuronic acid and α-l-guluronic acid	α-1, 4 glycosidic linkages	Biocompatibility, low toxicity, relatively low cost, Antioxidant, anti inflammatory, bioadhesive, antibacterial.	Stimulated monocytes, induces fibroblast proliferation and migration	[23]
4. Chitosan	Exoskeleton of crabs, molluscs, insects, fungal cell wall.	N-acetyl glucosamine	β-1, 4 glycosidic linkages.	Bioadhesive, biocompatible, biodegradable, hemostatic, mucoadhesive, anti inflammatory antimicrobial, wound healing.	Induces fibroblast and keratinocytes migration and proliferation.	[28]
5. Cellulose	Plant cell wall and bacteria	β-d-Glucose	β-1,4-glycosidic linkage	Biocompatible, and biodegradable, thermal properties, mechanical properties, rheological properties, barrier properties.	Retention of moisture, absorption of exudates.	[26]

6. Pullulan	fungus *Aureobasidiumpullulan*S.	maltotriose units.	α-(1 → 4) glycosidic bonds while maltotriose units are linked by α-(1 → 6) bonds.	Biocompatible, and biodegradable, adhesive property, antibacterial, antiviral, and antifungal agent, wound healing ability, anticoagulant.	Tissue engineering.	[29]
7. Gelatin	Bovine, porcine bones, skin, and ligaments of animals, certain types of seafood, such as fish skin and jellyfish.	Amino acids joined together in a chain.	β 1-4 amide linkage.	Biocompatible, and biodegradable, non-immunogenicity, low antigenicity, cost effective.	Induces fibroblast proliferation, induces secretion of ECM components by fibroblasts, chemotactic for macrophages.	[23]

and chemical entanglements due to presence of crosslinkers like H-bonding, ionic interactions and covalent bonding.

Their highly absorbent nature ensures presence of moist environment at the site of application. For the development of burn wound dressings, scaffolds can also be designed in a way where not much hydration is required to prevent microbial infection [7]. In this perspective, significant research has been performed on the use of biopolymers in the biomedical area.

1.2.4. Role of Peppermint (*Mentha piperita*) essential oil in wound healing

Peppermint essential oil (*Mentha piperita; PEO*) is known to have antimicrobial, antiviral, and antifungal activities against various types of bacteria and yeasts [30]. PEO has shown anti-inflammatory activity in the croton oil-induced mouse ear edema model due to its inhibitory effects on production of nitric oxide and prostaglandin E2 [31].

In this work we hypothesize that biopolymers-based scaffolds impregnated with PEO may be able to shorten pro-infammatory phase and accelerate the wound healing process. PEO contains many compounds, including menthol, menthon, isomenthol, limonene, cineol, menthyl acetate, beta-caryophyllene, menthofuran, terpinene, caron, pinene, Sabinene, β-pinene, tannin, etc. and are summarized in (**Table 3)** along with their therapeutic application [31].

Table 3: Major components of PEO accelerated wound healing **[30,31]**

Name	Attribute	Therapeutic benefits
Menthol	Minty flavor, odor & cooling effect	Antimicrobial, anti-inflammatory, anticandidal, carminative, antipyretic.
Menthone	Distinctive aromatic and misty fragrance with fresh camphoraceous, cooling and sweet attributes	Pesticide, analgesic, insecticide, stimulative, antiseptic and hypnotic
Menthofuran	Perfumery agent	Refreshing
1,8-Cineole	Fresh, strong, spicy, camphor like aroma Cooling taste	Bronchodilator, antiviral, antitussive, mucolytic, anti-inflammatory & cough suppressant
Limonene	Fragrance & flavor	Anti-carcinogenic &chemopreventive
Pulegol	Fainted aroma	Repellant and insecticidal
Isomenthone	Freshness	Uplifting
Neomenthol	Cooling, mentholic minty fragrance	Stimulating, refreshing, insecticide

1.2.5. Burn wound dressings

Dressings can be classified into passive and interactive dressings [32]. The different types of wound dressings used by victims during prehospital care are summarized in (**Table 4)**.

a) **Passive dressings**:- Simple products like gauze, no direct effect on the wound except protecting it e.g. protective clothes, gauzes etc.

b) **Interactive dressings**:- Most modern dressings, interact with the wound bed to provide optimum environment at the wound dressing interface e.g film, foams etc.

1.2.6. Desired properties of a burn dressing material

The materials that are most effective as burn wound dressings should mimic the extracellular matrix in wound healing function. The desirable properties in an ideal dressing should be [33] -

- **a.** Presence of moist wound environment
- **b.** Absorb extra wound exudates
- **c.** Maintain asepsis in wound
- **d.** Stimulate epithelialization and vascularization to enhance wound healing
- **e.** Adhesion to wound surface
- **f.** Non-toxic
- **g.** Biocompatible
- **h.** Biodegradable (at the site of wound on large burns)
- **i.** Provide ample oxygenation for wound healing

1. 3. Aim & Objectives

The aim of the present study is to develop and evaluate biopolymeric scaffolds for on-site management of casualties in the second degree superficial partial-thickness burn wounds.

Objective

The objective of this study is to explore blending of several combinations of natural biopolymeric formulations impregnated with suitable essential oil with tailored properties for on-site management of second degree superficial partial-thickness burn wounds.

The specific objectives as follows:

1. Selection of suitable biopolymeric candidates with a potential to be used in burn wound management.
2. Preparation, optimization, and characterization studies of different biopolymeric test scaffolds.
3. *In vivo* evaluation of optimized biopolymeric scaffolds for their efficacy and safety.
4. Animal dermal toxicity studies of optimized biopolymeric scaffolds.

Table 4: Different types of wound dressings used by victims during prehospital care[34]

Dressing category	Dressing type	Advantages	Disadvantages
Passive	Protective cloth, Gauges & Tulle	Easy to use, readily accessible in most clinics and surgical centers, Inexpensive	dry and do not control moisture levels, can adhere to wound bed triggering pain and mechanical trauma when removed, loses its efficiency when becomes saturated with exudate
Interactive	ns	Transparent self-adhesive sheets of polyurethane Forms a bacterial barrier but is gas and water vapor permeable, allowing for visualization of the wound, flexibility to use as a primary and or secondary dressing cover	Their non-absorbent properties can cause excess exudate accumulation, difficult to remove, which may disturb new epidermis
	Foams	Absorbent, moist healing environment, convenient over bony prominences or within exudative cavities	May be adherent or non-adherent, in which case a secondary film can be required, can adhere to wound if exudate dries
Advanced interactive	Hydrogels	Being available in different physical states: sheets, amorphous, and impregnated gauze,best for dry wounds as they have the ability to rehydrate and maintain a moist environment, comfortable Absorbent	Nonadherent, maceration of skin around wound Because of their high water content, their absorptive capacity is limited and would not be suitable for a wound with high exudate
	Hydrocolloids	Available as seets, pastes, and powders, moderately absorptive, easy to use, waterproof, stimulate autolytic debridement	Some of these dressings may adhere to the wound bed or be difficult to remove, The gel formed can be thick, yellow, and malodorous, chances of infection are high
	Alginates	highly absorbent making this dressing the best choice for highly exudative wounds	Not useful for dry wounds, may require frequent dressing change if lots of exudates
	Hydrofibers	Available in sheets and ribbons, highly absorbent	Nonadherent and need a secondary dressing to secure it, contraindicated for dry eschar, nonexudating wounds, third-degree burns, and heavy bleeding
Bioactive	Drug delivery systems	Drugs are loaded in different forms of dressing (e.g., hydrogels, films, electrospun fibers) delivering active substances, such as antimicrobials and antibiotics, which have a direct role in changing the chemical and cellular environment of the local wound, stimulating the healing cascade	Release profiles and rates to be optimized
	Biological dressings	Made from biological systems such as microorganisms, plants, and animals or chemically synthesized from biological materials such as starch, sugars, natural fats, and oils, collagen, gelatin, chitosan, hyaluronic acid based dressings promote fibroblast activity, and endothelial cells migration	Immunogenic response can be induced

1.4. Plan of work

1.4.1. Preformulation studies

a) Preparation of different combinations of biopolymeric microporous scaffolds: All test composite scaffolds shall be prepared by using freeze drying method. Varying concentrations (1-5%) of different biopolymers (viz., pullulan, gelatin, chitosan, alginate and carboxymethyl cellulose) shall be fabricated into scaffolds.

b) Optimization of suitable crosslinking agents: The hybrid mixture shall be crosslinked using suitable crosslinking agents (glutaraldehyde) after optimization. All test combinations shall be characterized using various physico-chemical parameters to identify the optimized combination for further evaluation.

b) Preparation of bioactive components (Peppermint oil; PEO)

Concentration of PEO shall be optimized for its impregnation into biopolymeric scaffolds. Optimization shall be performed by taking varying concentration of PEO (2-8%) in suitable carrier oil [85].

1.4.2 Formulation development

1.4.3. *In vitro* studies

a) Physical examination: The physical evaluation of fabricated biopolymeric scaffolds shall be studied on the basis of its texture, size, shape, appearance, uniformity, fragility and brittleness.

b) Fourier-Transform Infrared (FTIR) spectroscopy

c) *Fluid uptake ability of test scaffolds (Swelling studies):* The percentage of swelling (% swelling) of the scaffolds shall be determined by previously reported method:

Swelling index=[(Ws-Wd)/Wd] x 100

Where Ws is the swollen weight and Wd is the weight of the dried product.

d*) Thermogravimetric analysis (TGA)*

e*) Differential Scanning Calorimetry (DSC)*

f) Hemolytic activity test: Percent hemolytic index shall be performed by using the method [3] and calculated by following formula:

$$\% \text{ Hemolysis} = \{(OD_{test} - OD_{negative\ control}) / (OD_{positive\ control} - OD_{negative\ control})\} \times 100$$

Where, OD is the optical density.

g) In vitro biodegradation study: Degree of degradation shall be performed by using the method [35] and calculated by using the following formula:

$$\text{Degradation degree (D\%)} = (Wi - Wf)/Wf \times 100$$

Where, Wi = initial dry weight of scaffold, and Wf = weight of the dry scaffold after incubation in enzymatic solution.

h) Scanning electron microscopy (SEM): Difference in surface morphologies of various biopolymeric test scaffolds shall be investigated by SEM. Micrographs and the average diameter of pore size shall be assessed in each cross-sectional image of the scaffold samples.

i) Cytotoxicity studies: Biocompatibility studies shall be performed using MTT [3-(4,5-Dimethylthiazol-2- yl) -2,5-diphenyltetrazolium bromide] assay.

1.4.4. *In vivo* efficacy study

a) Creation of burn model: Second degree burn wound shall be created by heating a 2cm solid stainless steel bar to 110^0C -120^0C and then placing it for 10s over the shaved dorsum skin of the rats. Temperature of the metallic bar shall be standardized for all experimental groups using a visual IR thermometer (VT02/VT04A, Fluke, India). A local anesthetic (commercially available 2% lidocaine ointment) shall be applied topically 30 min prior to inflicting burn injury on the animals [3]**.**

b) Proposed animal groupings:

Rats shall be divided into four groups of 6 rats each;

Group I: negative control; i.e., given no treatment after inflicting burn wounds on them

Group II: positive control; shall be treated with commercially available silver sulfadiazine ointment (Silverex® SSD)

Group III: test control; shall be treated with peppermint oil only

Group IV: treatment group; shall be treated with optimized biopolymeric test scaffolds impregnated with peppermint oil.

c) Measurement of wound retraction: Measurement of wound retraction shall be doneon a daily basis using a digital Vernier callipers. Area of the wound at the start of experiment (Day 1) shall be considered as 100% to calculate the percentage reduction in the wound area. Formula for the calculation of the area of percentage wound retraction [36]:

Wound retraction rate (%) = (Initial wound size/wound size after an interval) x 100

d) Hematology studies: Blood samples shall be collected at different time intervals (1st, 7th, 14th & 21st day). Collected blood samples shall be evaluated for various parameters such as hemoglobin, red blood cells, white blood cells, packed cell volume, platelet counts, total differential counts, and erythrocyte sedimentation rate (ESR) etc.

e) Gram staining: Gram staining shall be performed within 3-4 day after creation of burn model to ascertain the presence of bacteria in infected samples and to compare the results between different experimental groups.

f) Histology evaluation: From each group, skin samples shall be harvested postoperatively at 1^{st}, 7^{th}, 14^{th} and 21^{st} day. Tissue histology shall be performed using hematoxylin and eosin staining and findings between different experimental groups shall be compared.

g) Immunohistochemistry (IHC) study: Epidermal growth factor (EGF) and fibroblast growth factor-2 (FGF-2) are known to play an important role in inducing collagen synthesis, reepithelialization, and skin regeneration [37]. IHC analysis shall be performed to compare the expression levels of these two growth factors using anti-EGF and anti-FGF 2 antibodies as an indicator of re-epithelialization and vascularization in all the animal groups.

h) Picrosirius red staining: Picrosirius red (PSR) staining shall be performed to visualize mature collagen in paraffin-embedded tissue sections. PSR stained collagen appears red in light microscopy [38].

i) Acute dermal toxicity study: Dermal irritation study for the test scaffold shall be carried out as per Organization for Economic Co-operation and Development (OECD) guideline 402.

1.4.5. Stability studies of test scaffolds

1.4.6. Compilation, interpretation and statistical analysis

1.5. Rationale of study

Combat related burn wounds are one of the most complex and painful injuries to treat and manage, both in military and civilian trauma situations. A majority of accidents with burn injuries in civilian set-up occur due to accidents at home or by virtue of occupational hazards

in industries. Patients with serious burns require immediate pre-hospital care so as to minimize morbidity and mortality. Treatment for burns is an intricate process and several potential drug formulations have been evaluated for this purpose [1]. Management of burns wounds encountered especially during military operations is a much more challenging task due to potentially greater number of simultaneous thermal/non thermal injuries inflicted in the battlefield. Previous data showed that 20% of the casualties encountered during combat operation are reported to sustain some form of burn injury [6]. The ability to provide initial on-site treatment at the point of burn injury can significantly reduce morbidity and mortality amongst personnel inflicted with variable degree of burns wounds.

Field medical kits used by military and paramilitary personnel in India mostly include cloth dressings. They are the most commonly used dressings for open wounds and minor injuries. The cloth gives the advantage of aeration to the wound but cannot maintain asepsis for long. Another similar traditional dressing includes gauze, which is a thin layer fabric of silk or cotton. A major reason for widespread use of these traditional dressings is its low cost and easy availability [39]. However, these dressings can adhere to the wound when the injury dries and damage the newly formed epithelium, thus slowing the healing of the injury. Another disadvantage of these dressings is the lack of sterility and inability to absorb exudates, leading to infection [40].Further, few of the topical agents like silver sulfadiazine or silver nitrate (Silverex) are commonly used but such agents are effective only when applied after proper cleansing and debridement, which unfortunately may not be possible during battlefield operation, thereby limiting their efficacy in such scenarios [41].

Though well-established procedures exist for management of burn wounds in a hospital set-up,no suitable indigenously developed formulation/ dressing is available for pre-hospital use. Some of these dressings are imported at an exorbitant cost by the Armed Forces and their availability in required numbers is often a concern. Therefore, on-site management of burn patients is one of the major challenges for military personnel during battlefield operations and there is an urgent need to focus for novel therapeutic interventions that can reduce the temporal gap between pre-hospital management and getting proper hospital-based treatment.

Figure 5: Various types of wound dressings available

In recent years, three dimensional scaffolds have become critical component of skin substitutes as they act as an extracellular matrix analog, guiding cell adhesion, proliferation and differentiation to create epidermal layers. Additionally, scaffolds provide a template around which new blood vessels form in a process called neovascularization. This process is necessary for viable dermal and epidermal layers at wound sites. Several different biopolymers have been used to create these scaffolds. Each polymer has it's unique characteristics affecting scaffold morphology, function of nearby cells, and physical properties of the skin substitutes.

The present work aims to compare the suitability and efficacy of pullulan based microporous s fabricated in combination with [alginate (Alg) / carboxymethyl cellulose (CMC), chitosan (CH) / gelatin (Gel)] biopolymers, and impregnated with peppermint oil (PEO), for management of second-degree skin burns in experimental rats. It is hypothesized that scaffolds made from pullulan in combination with other biopolymers as mentioned above shall not only serve as a substrate for skin regeneration, but addition of PEO shall further provide a local anesthetic and cooling effect at the wound bed during the healing process.

Part 2: Pharmaceutical approaches to augment pre-hospital management of venomous bites and their evaluation using lymphoscintigraphy

2.1. Introduction

Snakebite is a life-threatening condition that causes severe morbidity (~400,000/annum) and mortality (~138,000/annum) especially in tropical and subtropical countries [42]. In India alone, an estimated 200,000 persons per year fall prey to snake-bite, with an estimated fatality rate of 35,000-50,000 per year [43]. The incidence of envenomation is particularly high in tropical regions where snakes are abundant and human activities like field work and sleeping outdoors increase the risk of man-snake encounters. Epidemiological data showed that most

common sites of snakebite are foot (38%) and hands (31%). Others exposed sites are arms (16%), head and face (7%) and trunk (1%) [44] (**Figure 6**)

Moreover, it is one of the major problems among military and paramilitary personnel deputed in North-east and other naxal-affected areas. In 2017, the World Health Organisation (WHO) re-classified snakebite as a priority neglected tropical disease and flagged it as a severe global public health crisis and reduces the cases of morbidity and mortality, by the end of year 2030, by improving existing treatments, repurposing drug therapeutics, develops novel/small candidates and educates local communities regarding improvement in pre-hospital treatment [45]. Snake venoms are complex cocktails of pharmacologically active proteins, polypeptides, enzymes, non-protein component and small organicand inorganic molecules [46].

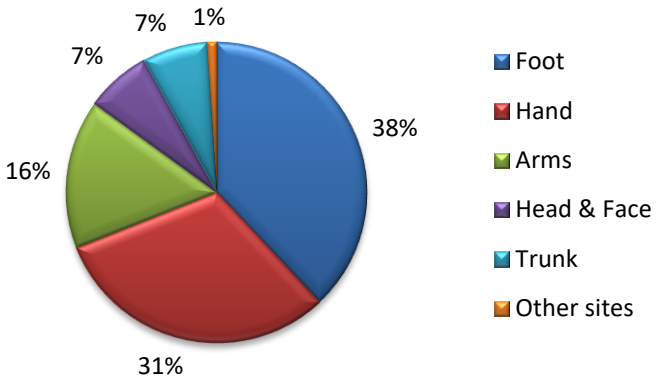

Figure 6: Pie chart of the sites of snakebite[4]

In general, the toxic components of the snake venom are usually proteins, which are classified into two categories:**(a) High molecular weight (HMw) components**(>20kDa), comprising of snake venom metalloproteinases (SVMPs), Snake venom serine proteases (SVSPs), L-amino acid oxidases (LAAOs), acetylcholinesterase (AChE) and hyaluronidases, and **(b) Low molecular weight (LMw) components** (<20kDa), comprising mainly of secretoryphospholipases-A2 (sPLA2s), 3-Finger toxins (3FTXs), Kunitz peptides, disintegrins, cysteine-rich secretory proteins, C-type lectins, and natriuretic peptides [47]. After snakebite these toxic components travel in the body by different routes and manifest multiple local and systemic effects. Local tissue effects are mainly seen due to myotoxic and cytotoxic components resulting in edema, pain, tissue necrosis, blistering, hemorrhage, dysgeusia, numbness and tingling proximal to the bite site, whereas systemic effects are generally more serious and may lead to coagulopathy, neurotoxicity, myotoxicity, vasculotoxicity, cardiotoxicity, acute kidney or respiratory failure and death [48,49].

Nearly, 75% of envenoming cases occur far from primary hospital settings leading to fatalities due to delayed treatment, particularly in rural settings [50]. Pre-hospital management in such

cases is a challenging task due to requirement of stringent storage conditions for anti-venoms, poor health services, lack of adequate training for snakebite management, and inappropriate field/ first aid measures [51]. Currently, on-field first-aid measure include use of pressure bandage immobilization (PBI), which has sub-optimal efficacy in managing snakebite casualties, even if applied by trained personnel [52]. Nonetheless, several reports argue against the use of PBI in viper's snake bites because of extensive local necrosis [43].

Based on these considerations, there is an urgent need for non-immunological therapeutic interventions that could be self-administered immediately after the snakebite, alone or in tandem with PBI, to reduce the mortality caused by snakebite. Notably, the snakebite victims usually experience distress and unconsciousness making per-oral delivery difficult [53]. Thus, combination of drugs administered through convenient, self-administrated topical route either to delay absorption and transit of the venom into the circulation, thus could be useful for pre-hospital management following snake bite.

2.2. Literature Review

As per literature poisonous snakes can be divided into three families [43]:-

1. ***ELAPIDAE***– Cobras, Kraits, Mambas and Tiger snakes.
2. ***VIPERIDAE***- (i) Viperinae: Russels' Viper, Saw-scaled Viper
   (ii) Crotalinae: Pit viper
3. ***HYDROPHIDAE*** – Sea Snakes

Snake venom is made up of several hundreds of peptides and proteins. However, SVMPs, SVSPs, sPLA2s and 3FTX are mainly accountable for its toxic effects including hemotoxic/vasculotoxic effects such as hemorrhage and coagulopathy, cytotoxic/ myotoxic effects such as necrosis and inflammation, and neurotoxic effects such as severe pain and paralysis [54,55]. Both, SVMPs and SVSPs are high molecular weight proteins; abundant in viperid snake venoms, with predominant hemotoxic and cytotoxic effects e.g. *Daboia russelii* (Russels' viper), *Echis carinatus* (saw-scaled viper) and pit viper (rattlesnakes) [56]. The venom metalloproteinases are known for their hemorrhagic and extracellular matrix-degrading activities. The hemolytic activity is mediated through cleavage of basement membrane, detachment of endothelial cells from the basement membrane, and rupturing of the capillary walls [57,58]. In addition, SVMPs exhibit fibrinolytic/fibrinogenolytic activity causing inhibition of platelet aggregation, coagulopathy, inflammation and edema, as in case of *Protobothrops mucrosquamatus* (Brown Spotted pit viper) [59]. SVSPs, on the other hand exhibit their hemotoxic effects by fibrinolytic/fibrinogenolytic activities, perturbations of blood coagulation, platelet aggregation and activation of coagulation factors as in cases of

pit vipers (Agkistrodon, Bothrops, Lachesis and Trimeresurus), true vipers (Bitis and Cerastes) and colubrids [60]. These mechanisms significantly contribute to the establishment of coagulopathy and external and internal bleeding [61]. Some of the SVSPs are also known for kininogenase activity, whereby they activate release of endogenous mediators (bradykinin) resulting in increased vascular permeability, edema, blood pressure and cardiovascular effects [62]. Notably, following a snake bite, SVMPs (size, 20-110 kDa) [8,33] and SVSPs (size, 26-67 kDa) [63] are predominantly absorbed into the lymphatic system and following their lymphatic transit they trespass to the systemic circulation to produce toxic effects [64].

Low molecular weight snake venom sPLA2s (size, 13-15 kDa) and 3FTxs (Size, ~6-9 kDa) are abundant in *elapidae* venomous snakes [47,65]. These small toxin proteins have affinity for a wide spectrum of targets and exert diverse toxicological effects. sPLA2s play an important role in exhibiting myotoxic effects e.g. *Naja species[Naja Naja* (cobras) and *Najas umatrana*, *Naja kaouthia*) (southeast Asian Cobras] and neurotoxic effects e.g. *Bungarus caeruleus* (kraits), *Naja species (cobras), Oxyuranus species* (Taipans), *Micrurus species* (coral snakes) and *Notechis scutatus* (Tiger snakes) [65]. The myotoxicity and necrosis caused by sPLA2s are mediated through enzymatic cleavage of cell membrane phospholipids and also through mechanism independent of its catalytic activity, triggered through its high affinity interaction with target protein e.g. *Hydrophiinae* (sea snakes) [66].

Further, sPLA2s exhibit pre-synaptic neurotoxic effects by binding to the motor nerve terminals, leading to exocytosis of the synaptic acetylcholine (ACh) vesicles, depletion of ACh, inhibition of the neuromuscular junction transmission and muscle paralysis. sPLA2s also promote edema, inflammation and hyperalgesia through block of neuromuscular transmission [67]. Interestingly, the pre-synaptic binding of toxin to the nerve terminal is irreversible; however the effects develop following a latency period of 20–60 minutes [68]. The snake venom composition and it's toxicity profile are summarized in **(Figure 7)**

On the other hand, non-enzymatic 3FTXs have varied targets and diverse biological activities. 3FTXs bind to post-synaptic nicotinic cholinergic receptors (α-neurotoxins) and causes flaccid or spastic paralysis due to the inhibition of AChE and ACh receptors [69]. 3FTxs also exert cytotoxicity by disorganizing cellular membranes [44], altering cardiac rate through modulation of α- and β-adrenoreceptors [e.g. *Ophiophagus hannah* (king cobra) and *Naja oxiana* (caspian cobra)], and altering homeostasis through inhibition of platelet aggregation and presynaptic neurotoxicity (β-neurotoxins) [e.g. *Dendro aspispolylepis* (mambas)] [70]. Unlike proteases, the LMw (sPLA2s and 3FTXs) are rapidly and directly absorbed into the systemic circulation hence making the therapeutic management difficult, particularly during time lapse between the bite and accessibility to a health care [48].

Previous published reports suggest that Ca^{+2} ions function as signaling molecules that control contraction and thereby transit of venom toxicants through lymphatic smooth muscles [71]. Nifedipine, a calcium channel blocker, is used to treat high blood pressure and angina by inhibiting contraction of smooth muscles [52].

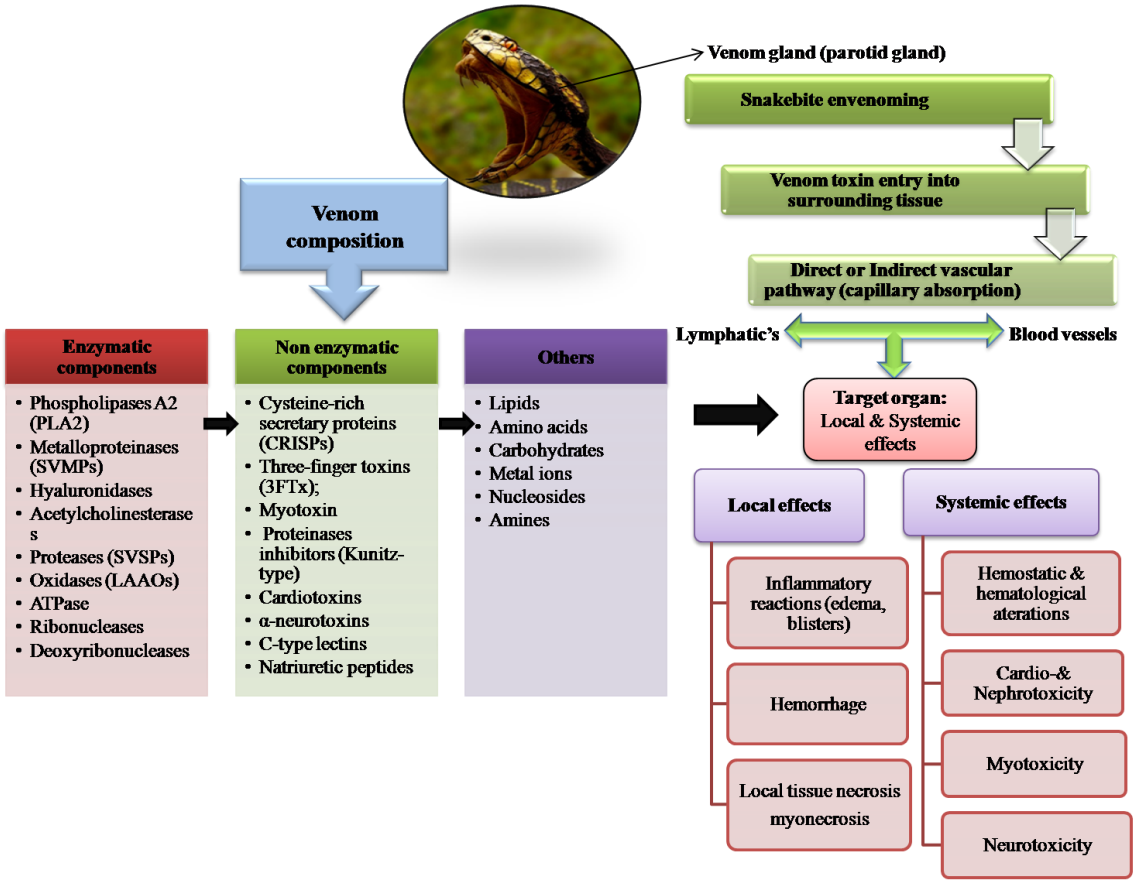

Figure 7: Snake venom composition and its toxicity profile [68]

From the result of an *ex-vivo* study, it is evident that nifedipine inhibits contractile activity of human lymphatic vessels [72]. Also, we found evidence that topical nifedipine (0.5%) is effective in treatment of anal fissures through Ca^{+2} mediated relaxation of anal smooth muscles [73]. Nitric oxide (NO) donor like glyceryl trinitrate ointment (GTNO, 0.2% w/w) and sodium nitroprusside increase the foot to groin transit time of lymph through the stimulation of the soluble guanylate cyclase, formation of cyclic GMP and activation of protein kinase G, resulting in reduction of intracellular Ca^{2+} concentration and decrease in the sensitivity of contractile system to Ca^{2+}. The net effect is both smooth muscle relaxation and an inability to pace the muscle, which disables the lymphatic pump. Another drug that could be used in conjugation with nifedipine (0.3%) is lidocaine (1.5%) [74]. Lidocaine is widely used local anaesthetic with rapid onset of action (< 2 min) and long duration (~ 2 h). Lidocaine might be able to decrease lymphatic transit through its vasodilation action mediated by blocking nerve (i.e. Na^{+}) -mediated action potentials [75]. Another advantage

of using lidocaine is that it shall also relieve local pain and analgesia, experienced by snake bite victims [52]. The use of topical lidocaine in management of pain and analgesia has been penned in several previous reports [76]. As a result, we hypothesized that low dose topical nifedipine and lidocaine could decrease lymphatic transit of HMw toxins by lymphatic smooth muscle relaxation. This translates into increased lag time for venom proteins to reach systemic circulation and exerts toxic effects, thus increasing mean survival time.

LMw venom proteins require inhibitors to either prevent or delay the emergence of toxic effects. Based on the evidence we found that varespladib and neostigmine could be promising inhibitors of sPLA2s and 3FTXs, respectively [77]. Varespladib, an inflammatory drug, has been investigated for the treatment of acute coronary syndrome and acute chest syndrome but has shown limited efficacy. It locks inflammatory cascade by inhibiting sPLA2, which promotes inflammation and the immune response by breaking down phospholipids and releasing arachidonic acid (AA) [78]. Recent studies suggest that varespladib is a potent and broad spectrum inhibitor of snake venom sPLA2s [50]. Several chromogenic assay based studies have suggested that varespladib could inhibit the sPLA2 activity of large arrays of snake venoms at nanomolar and picomolar amounts [77]. An *in vivo* study has demonstrated remarkable survival benefit of using varespladib against *Vipera berus* and *Micrurus fulvius* venom and suppression of sPLA2s activity in rats challenged with lethal doses of venom. Varespladib has also been shown to increase mice survival time from 3h to 24h and attenuate the neurotoxicity of snakes caused by *Bungarus indanus*, *Bungarus multicinctus* and *Bungarus fasciatus* venoms [79]. A part from their promising effects against neurotoxins, varespladib has demonstrated efficacy against hemotoxic snake venoms and also shown to neutralize the coagulopathy. Varespladib has recently received orphan drug status by USFDA for snakebite envenomation [80]. Most of the reports highlighting efficacy of varespladib (prodrug) against sPLA2s have utilized oral therapy [81]. However, in a very recent study using mouse model of edema, varespladib has shown anti-inflammatory activity equivalent to marketed immunosuppressant, justifying the topical use of varespladib [82].

Acetylcholinesterase inhibitors such as edrophonium and neostigmine are thought to subside the neuromuscular block from neurotoxic 3FTXs by elating the amount of acetylcholine at the neuromuscular junction as they do in the treatment of myasthenia gravis [83]. Studies in animals strongly suggest that treatment with acetylcholinesterase/cholinesterase inhibitor(s), greatly reduces mortality from neurotoxic envenomation [84], and clinical studies have shown improved clinical parameters and functions [85]. Neostigmine has rapid onset (< 1min) and longer duration of action (20-30 min) and is more potent (0.04 mg/ kg), reliable and safer than edrophonium [86]. Several clinical studies have demonstrated the efficacy of

neostigmine is part of standard treatment guidelines of various countries, with or without antivenom, in resuscitating snake bite victims [87]. Although the use of neostigmine is included in the WHO guidelines, its use is still conservative as the results could be variable in different patients [87].It is recommended to use it along with atropine to improve neuromuscular transmission in patients with post-synaptic neurotoxicity caused by snakes such as cobras and Australasian death adders [88].

Since the snake venom toxins produce considerable distress in snakebite victims, our aim is to use suitable pharmacological interventions using a combination of drugs, to modulate the lymphatic flow rate so as to delay venom toxins reaching the systemic circulation through the lymphatic system thereby augmenting the present pre-hospital management techniques.

2.3. Aim & Objectives

The present study aims to evaluate the use of suitable therapeutic interventions to augment existing technology for pre-hospital management of venomous bites. The aim is to investigate the effect of a few topical pharmacological agents in modulating the lymphatic flow so as to delay venom toxins reaching the systemic circulation through the lymphatic system. The work is based on the hypothesis and some previously published reports that snake venoms contain large toxin molecules (>20 KDa), which cannot enter the blood stream directly and are absorbed and transported by lymphatic vessels into the systemic circulation [52,89].

The study shall have the following objectives:

1. Screening, optimization, and characterization of suitable topical formulation(s) comprising of agents known to modulate peripheral lymphatic flow rates.
2. Selection and analysis of a suitable radiopharmaceutical namely (^{99m}Tc-Sulphur Colloid, ^{99m}Tc-Phytate, ^{99m}Tc-Human serum albumin, which can be used as ‘mock-venom’ in studying modulation in lymphatic transit behavior using gamma scintigraphy (also known as lymphoscintigraphy).
3. Demonstrate the effect of optimized formulation(s) on lymphatic transit times through lymphoscintigraphy studies in preclinical models by using selected radiopharmaceutical as ‘mock-venom’.

2.4. Plan of Work

2.4.1. Pre-formulation studies

a) Selection of APIs and their solubility in solvents like Ethanol, DMSO etc.

b) Preparation of topical formulation using different API combinations (Selection of vehicle/base: cream, gel and ointment)

c) Optimization of formulation(s) (single API/ combination): Optimization of all the formulations shall be performed by evaluating the effect of different concentrations of API and vehicle (base) on the stability, viscosity, and any visual changes in the formulations. After optimization, stable formulations, which provide acceptable appearance, pH, spreadibility and viscosity shall be chosen for further evaluation. The main aim of study is to evaluate how formulation parameter such as concentration and physical state of drug, formulation type influences the permeation of drug (APIs) through the skin.

2.4.2. Formulation development

2.4.3. In vitro studies

a) Physicochemical evaluation of topical formulation, which will include:

i. Organoleptic characteristics
ii. Melting point
iii. Spreadability
iv. Hardness
v. pH values
vi. Viscosity measurement
vii. Homogeneity
viii. Drug content
ix. Drug combatibility studies: SEM, DSC & Hot stage microscopy
x. *In vitro* diffusion study: Franz diffusion to check drug release study
xi. *Ex vivo* permeation study
xii. Skin irritation study
xiii. Physical stability study

b) Characterization studies

i. Differential scanning calorimetry (DSC)
ii. Thermo gravimetric analysis (TGA)
iii. Dynamic vapour sorption studies
iv. Scanning electron microscopy

2.4.4. *In vivo* studies

i. Selection and analysis of a suitable radiopharmaceutical e.g., (^{99m}Tc-sulphur colloid, ^{99m}Tc-Phytate, ^{99m}Tc-Human serum albumin), which can be used as mock venom in studying modulation in lymphatic transit behavior using gamma scintigraphy.

ii. Demonstrate the effect of optimized formulation(s) on lymphatic transit times through lymphoscintigraphy in preclinical models by using selected radiopharmaceutical as mock venom in vivo

Following parameters shall be studied during lymphoscintigraphy study:

- *Lymph transit times:* This will give in vivo information about the efficacy of topically applied formulation(s) in delaying lymphatic flow and its comparison with control group.
- *Comparative evaluation and rate of radiotracer trapping time in abdominal lymph nodes and liver*

iii) Dermal toxicity: Assessment of toxicity of the test formulations as per OECD guidelines.

2.4.4. Stability studies

2.4.5. Compilation and statistical analysis

2.5. Rationale of study

Lymphatic vascular system is crucial for maintaining fluid balance, lipid absorption, immune-surveillance and help larger molecules (>20kDa) enter into the systemic circulation. Our hypothesis is based on the fact that molecular weights of snake toxins molecules are generally large and therefore they first transverse into the lymphatic vessels with aid of lymphatic pump, before entering the systemic circulation [89]. Depending on their molecular weight, high molecular weight (SVMPs, SVSPs) and low molecular weight (sPLA2, 3FTXs) venom toxins follow different routes for entering into the systemic circulation. HMw toxins like SVMPs and SVSPs enter the bloodstream through lymphatic system, whereas LMw toxins like sPLA2 and 3FTXs enter the systemic circulation directly and manifest their toxic effects [48]. Considering that the lymphatic system plays a crucial role in transportation of 'high molecular weight' toxin molecules into the circulation, and the fact that pharmacological interventions can be designed to modulate lymphatic flow rates, the present study has been designed with an aim to augment existing technology for pre-hospital management of

venomous bites. If successful, it may help in extending the 'golden period' required for evacuation of such victims to a nearby primary health centre for getting conventional antivenom treatment. This may help in reducing morbidity and mortality associated with snake envenomation among army personnel. Though single pharmacological agents like NO donors, Ca^{2+} ion channel inhibitors etc. have been studied for modulating lymphatic flow rates, we propose to use a combination of drugs formulated into a suitable delivery system for early and sustained action. The hypothetical mechanistic approach highlighting proposed pharmacological interventions are summarised in (**Figure 8).** Another novelty of the work is the use of lymphoscintigraphy in evaluation of this therapeutic intervention. This will provide real time in vivo information w.r.t. modulation in lymphatic transit times between different experimental groups.

Lymphoscintigraphy is an advanced functional imaging technique used for mapping the lymphatic flow rate and lymph node visualization commonly employed in clinical settings. For lymphoscintigraphy, radiopharmaceutical is directly administered to humans either subcutaneously or intradermally, which then demonstrates the movement of radiotracer through lymphatic channels into the lymph nodes and further into systemic circulation [90]. Numerous radiopharmaceutical agents preferred for lymphoscintigraphy are Technetium-99m (^{99m}Tc) antimony trisulfide colloid, ^{99m}Tc-sulfur colloid (SC), ^{99m}Tc-nanocolloid, ^{99m}Tc-human serum albumin (HSA) and ^{99m}Tc Phytate (Phy), depending upon their availability in different regions of the world. Although the toxins of most front-fanged snake's family vary considerably in molecular size and weight, they enter the systemic circulation via the lymphatic's, unless there is direct penetration of the blood vessel [91]. Similar transit behavior of the radiopharmaceutical is seen in lymphoscintigraphy studies, where particles in the range of 10-100nm are transported from interstitial tissues through lymphatic system, while those <10nm reaching the bloodstream directly. Therefore, although not a fool-proof model, lymphoscintigraphy is a safe and sensitive method, which has been used to study lymphatic flow rates [92].

We believe that demonstration of delaying lymphatic transit could improve mean survival time following snake envenomation, thereby opening up a new avenue for research. Since the snake venom toxins produce considerable distress in snakebite victims, our approach is based on pharmacological modulation of the intrinsic lymphatic pump using combination of drugs and assessing venom entry into the circulation after formulating and applying a suitable topical ointment/ gel formulation and deliver it through a convenient and self-administrable route for better pre-hospital management of such cases with an aim to delay onset of systemic venom toxicity. We also believe that evaluation of adjunct method along with test formulations may possibly impede venom transport

through circulatory system and can be beneficial in reducing mortality among envenomized soldiers during evacuation process

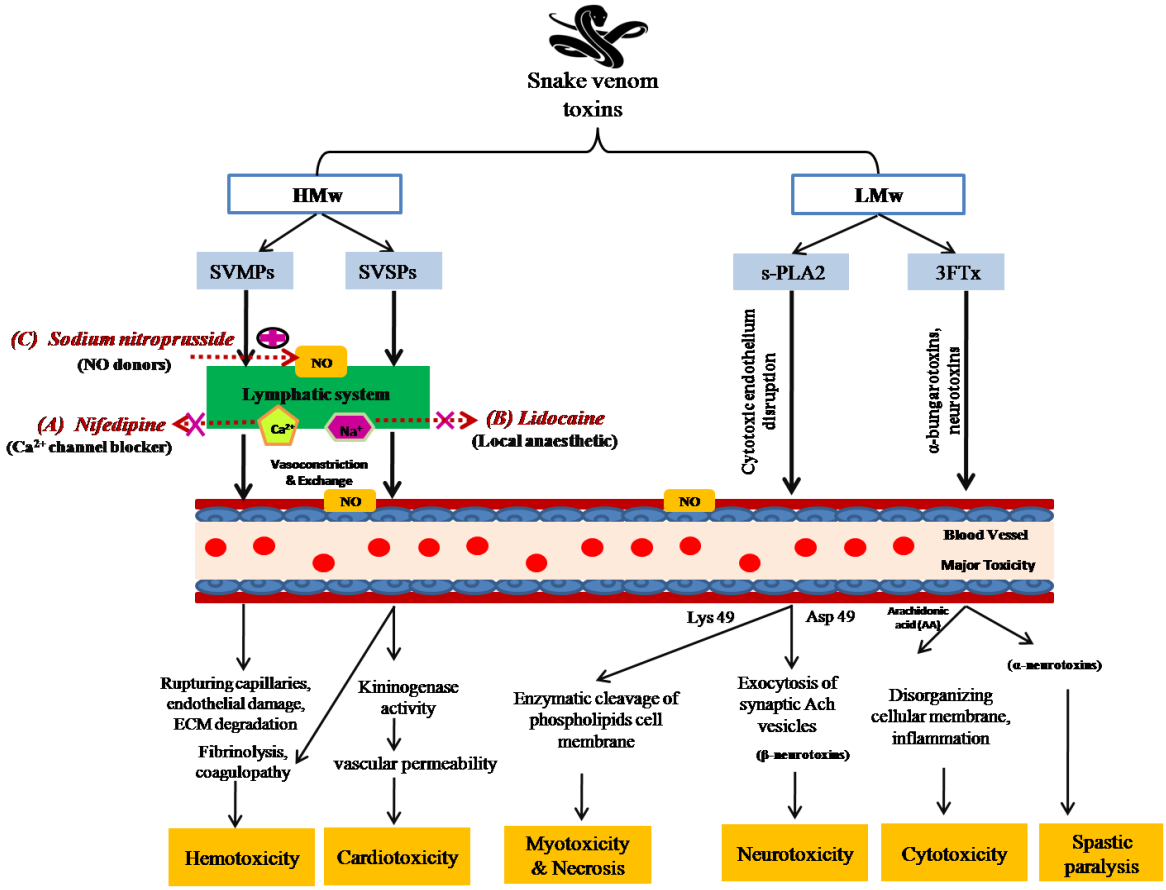

Figure 8: Schematic diagram depicting pathogenesis pathway and potential HMw pharmacological targets for pre-hospital management of snake envenoming (A) Calcium ion-channel blockers: Nifedipine; (B) Local anaesthetic agent: Lidocaine;(C) NO donors: Sodium Nitroprusside.

(Abbreviations: Lys 49: Lysine residues; Asp 49: aspartic acid; AA: Arachidonic acid; HMw: High molecular weight; LMw: Low molecular weight)

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